Amendments to the Claims:

This listing of claims will replace all prior versions and listings of claims in the application:

Listing of Claims:

Claim 1. (Currently amended) A method of ~~detecting the presence of~~ counting a single ~~copy~~ molecule of a target nucleic acid in a sample, said method comprising:

(i) detecting an optical characteristic of a first quantum dot and a second quantum dot attached to said single ~~copy~~ molecule of said target nucleic acid, wherein said first quantum dot and said second quantum dot are distinguishable; and

(ii) resolving said optical characteristic of said first quantum dot and said second quantum dot attached to said single molecule of said target nucleic acid from an optical characteristic of a quantum dot not attached to said single molecule of said target nucleic acid, thereby ~~detecting~~ counting said single ~~copy~~ molecule of said target nucleic acid.

Claim 2. (Previously presented) The method as in claim 1, further comprising quantitating the target nucleic acid by analyzing the detected optical characteristic.

Claim 3. (Previously presented) The method as in claim 1, further comprising transcribing the target nucleic acid.

Claim 4. (Previously presented) The method as in claim 3, wherein the target nucleic acid comprises DNA and transcribing comprises using a primer which anneals to a conserved region of the DNA and transcribes a polymorphic region of the DNA when extended.

Claim 5. (Previously presented) The method as in claim 4, wherein the primer is biotinylated and the transcribing step produces biotinylated DNA.

Claim 6. (Previously presented) The method as in claim 3, further comprising binding the transcribed target nucleic acid to a substrate.

Claim 7. (Original) The method as in claim 6, wherein the substrate comprises a streptavidin coated surface, support, plate or slide.

Claim 8. (Previously presented) The method as in claim 6, further comprising removing unbound portions of the target nucleic acid.

Claim 9. (Previously presented) The method as in claim 6, further comprising probing the bound target nucleic acid using a sequence-tagged hybridization probe.

Claim 10. (Original) The method as in claim 9, wherein the target comprises DNA having at least one point mutation and the probing comprises binding the probe to said at least one point mutation of the DNA.

Claim 11. (Original) The method as in claim 9, wherein the target comprises wild type DNA and the probing comprises binding the probe to the wild type DNA.

Claim 12. (Original) The method as in claim 9, further comprising removing non-specifically bound probe.

Claim 13. (Original) The method as in claim 9, wherein each quantum dot has an attached oligonucleotide tag and labeling comprises binding each tag with a complementary sequence of each sequence-tagged hybridization probe.

Claim 14. (Original) The method as in claim 13, further comprising removing unbound quantum dots.

Claim 15. (Previously presented) The method as in claim 13, wherein detecting comprises scanning the substrate with resolution capable of detecting an optical characteristic of a single quantum dot.

Claim 16. (Previously presented) The method as in claim 15, further comprising quantitating the target nucleic acid by analyzing the detected optical characteristic, wherein analyzing comprises counting the number of quantum dots within an area of scanned substrate.

Claim 17. (Currently amended) A method of ~~detecting the presence of~~ counting a single ~~copy~~ molecule of a target nucleic acid in a sample, said method comprising:

(i) detecting an optical characteristic of a first quantum dot and a second quantum dot attached to said single ~~copy~~ molecule of said target nucleic acid, wherein said first quantum dot and said second quantum dot are distinguishable;

(ii) resolving said optical characteristic of said first quantum dot and said second quantum dot attached to said single molecule of said target nucleic acid from an optical characteristic of a quantum dot not attached to said single molecule of said target nucleic acid, and

(iii) quantitating the target nucleic acid by analyzing the detected emitted fluorescence,

thereby ~~detecting~~ counting said single ~~copy~~ molecule of said target nucleic acid.

Claim 18. (Currently amended) A method of ~~detecting the presence of~~ counting a single ~~copy~~ molecule of a target nucleic acid in a sample, said method comprising:

(i) transcribing said single ~~copy~~ molecule of said target nucleic acid using a primer comprising an immobilizable label to form an immobilizable target nucleic acid;

(ii) immobilizing said immobilizable target nucleic acid on a solid support to form an immobilized target nucleic acid;

(iii) contacting said immobilized target nucleic acid with a sequence-tagged hybridization probe comprising a sequence complementary to a portion of said target nucleic acid;

(iv) detecting an optical characteristic of a quantum dot conjugate comprising a first quantum dot, a second quantum dot, and a nucleic acid sequence complementary to a portion of said sequence-tagged hybridization probe, wherein said first quantum dot and said second quantum dot are distinguishable;

(v) resolving said optical characteristic of said quantum dot conjugate from an optical characteristic of a quantum dot conjugate not attached to said

immobilized target nucleic acid, thereby ~~detecting~~ counting said single ~~copy~~ molecule of said target nucleic acid.

Claim 19. (Previously presented) The method as in claim 1, wherein said optical characteristic is detected by coincidence detection.

Claim 20. (Cancelled)

Claim 21. (Cancelled)

Claim 22. (Cancelled)

Claim 23. (Previously presented) The method as in claim 1, wherein said first quantum dot and said second quantum dot are distinguishable by an optical characteristic which is a member selected from the group consisting of fluorescence spectrum, fluorescence emission, fluorescence excitation spectrum, ultraviolet light absorbance, visible light absorbance, fluorescence quantum yield, fluorescence lifetime, light scattering and combinations thereof.

Claim 24. (Previously presented) The method as in claim 1, wherein said optical characteristic is fluorescence.

Claim 25. (Previously presented) The method as in claim 1, wherein said first quantum dot and said second quantum dot are visually distinguishable as a first color and a second color, respectively.

Claim 26. (Previously presented) The method as in claim 25, wherein said first color and said second color combine to form a third color that is visually or electronically distinguishable from both said first color and said second color.

Claim 27. (Previously presented) A method of selecting a mutant DNA away from a wild type DNA, said method comprising:

contacting mutant DNA attached to a first and a second sequence-tagged hybridization probe with a first and a second oligonucleotide tag comprising a sequence complementary to said first and second sequence-tagged hybridization probes and conjugated to

a first quantum dot and a second quantum dot, wherein said first quantum dot and said second quantum dot are distinguishable;

contacting wild type DNA attached to a third and a fourth sequence-tagged hybridization probe with a third and fourth oligonucleotide tag comprising a sequence complementary to said third and fourth sequence-tagged hybridization probes and conjugated to a third quantum and a fourth quantum dot, wherein said third quantum dot and said fourth quantum dot are distinguishable; and

detecting an optical characteristic of the quantum dots, whereby detection of said optical characteristic of said first quantum dot and said second quantum dot detects the mutant DNA and detection of said optical characteristic of said third quantum dot and said fourth quantum dot detects wild type DNA.

Claim 28. (Previously presented) The method as in claim 27, wherein said first quantum dot and said second quantum dot are distinguishable by an optical characteristic which is a member selected from the group consisting of fluorescence spectrum, fluorescence emission, fluorescence excitation spectrum, ultraviolet light absorbance, visible light absorbance, fluorescence quantum yield, fluorescence lifetime, light scattering and combinations thereof, and

wherein said third quantum dot and said fourth quantum dot are distinguishable by an optical characteristic which is a member selected from the group consisting of fluorescence spectrum, fluorescence emission, fluorescence excitation spectrum, ultraviolet light absorbance, visible light absorbance, fluorescence quantum yield, fluorescence lifetime, light scattering and combinations thereof.

Claim 29. (Previously presented) The method as in claim 27, wherein said first quantum dot and said second quantum dot are distinguishable by an optical characteristic which is fluorescence; and

wherein said third quantum dot and said fourth quantum dot are distinguishable by an optical characteristic which is fluorescence.

Claim 30. (Previously presented) The method according to claim 27, wherein said first quantum dot, said second quantum dot, said third quantum dot, and said fourth quantum dot are

visually or electronically distinguishable as a first color, a second color, a third color, and a fourth color respectively.

Claim 31. (Previously presented) The method according to claim 30, wherein said first color and said second color combine to form a visually or electronically distinguishable color different from both said first color and said second color, and

wherein said third color and said fourth color combine to form a visually or electronically distinguishable color different from both said third color and said fourth color.

REMARKS/ARGUMENTS

Applicants wish to thank Examiner Forman for extending the courtesy of the telephonic interview held on April 30, 2003 with Applicants' representatives, Jeffry Mann and Todd Esker.

I. Status of the Claims

After entry of this amendment, claims 1-19, 23-31 are pending. Claims 1 and 17-18 have been amended. Claims 20-22 have been cancelled. The amendments do not introduce new matter or raise new issues that would require further consideration and/or search.

II. The Invention

The invention provides assays that allow for the counting of a single molecule of a target nucleic acid. The single molecule is either directly or indirectly labeled with two or more differently colored semiconductor nanocrystals, or quantum dots. Quantum dots produce bright and tunable fluorescence that can be readily counted. Assays based on the counting of two or more differently colored quantum dots on a single molecule of interest provide highly sensitive assays in which the fluorescence from quantum dot-bound single molecules is spatially resolved from background noise and from unbound quantum dots.

Earlier methods of detecting a target species of interest are known as "ensemble detection". Below a threshold concentration or density, ensemble detection methods saturate, and are unable to differentiate the total signal of quantum dot-bound targets from the background noise and from unbound quantum dots (page 16, lines 10-16). Thus, the signal detected in ensemble detection is the total emission intensity for a population of a target species collected over an entire assay region. Following detection, the detected signal is compared with the emission intensities of known target species concentrations or densities, in order to quantify the amount of a target species of interest.

Single molecule counting is a departure from these earlier methods of quantifying the amount of the target nucleic acid. Single molecule counting is performed at concentrations or densities where individual molecules can be spatially resolved from background noise. Thus, the signal detected is the actual, directly observed signal of quantum dots attached to an

individual molecule of the target nucleic acid. While ensemble detection measures the total emission intensity of a population, single molecule counting involves counting optical characteristics produced from quantum dot-bound individual molecules. Because it is based upon direct observation, single molecule counting does not require the extra comparison step necessary for ensemble detection. Therefore, single molecule counting provides a less complicated and more sensitive method of quantifying the amount of a target nucleic acid than ensemble detection.

III. Support for the Amendments

Support for the amendments to the claims can be found throughout the specification, the drawings, and the claims as originally drafted.

During the April 30th telephonic interview, the Examiner suggested that adding the resolving step limitations of claims 20-22 to the Applicants' independent claims would add to the strength of Applicants' patentability arguments. Applicants have taken the Examiner's advice and amended claims 1 and 17-18 to include limitations from claims 20-22. Support for these amendments are found on page 23, lines 8-11, as well as in claims 20-22 as filed. Applicants thank the Examiner for her guidance.

Claims 1 and 17-18 have been amended to substitute the word "copy" with the word "molecule". The present invention discloses methods for increasing the sensitivity, specificity and dynamic range of assay systems based upon the capture of a "target species" with an affinity moiety. (p. 13, lines 5-8). "Target species" can refer to the counting of an individual "copy", in a method referred to as "single target counting". (p. 14, lines 30-31). In "single target counting", the detected signal represents an individually bound target "molecule" in those instances where the target "molecule" is separated from another target "molecule" by a distance which allows for proper resolution (p. 16, lines 2-5). "Single target counting", often referred to as "single copy counting" in this application (p. 16, line 8; p. 17, lines 3-5; p. 17, lines 9-11), is contrasted with ensemble counting (p. 16, line 27; p. 17, line 1; p. 17, line 4; p. 17, line 10). In ensemble counting, the detected signal represents the average emission intensity of a population of molecules (p. 15, line 30 to p. 16, line 2). Because "single target counting"/"single copy

counting" is interchangeably defined as counting an individual copy and representing an individually bound target molecule, the words "copy" and "molecule" have similar meanings in the application. Therefore, exchanging "molecule" for "copy" in claims 1 and 17-18 is supported by the specification.

Therefore, no new matter is introduced with this amendment.

IV. Response to Restriction Requirement

Claims 27-31 are allegedly directed to an invention that is independent and distinct from claims 1-26. Since an action on the merits has been issued for claims 1-26, these claims were provisionally elected for prosecution by the Examiner and claims 27-31 were withdrawn. Because Applicants do not believe that the search requirements in this case constitutes an undue burden, Applicants respectfully traverse the Restriction Requirement.

V. Response to Claim Rejections

Under 35 U.S.C. § 102(e)

To maintain a *prima facie* case of anticipation, the Examiner must demonstrate that each and every element as set forth in the claim is either expressly found or is inherently described in a single enabling prior art reference. The identical invention must be shown in as complete detail as is contained in the ...claim. See MPEP § 2131. In addition, a prior art reference is presumed to enable one of skill in the art to make and use the invention without undue experimentation. See MPEP § 2121. Factors used to determine undue experimentation include, but are not limited to: (i) the amount of direction provided by the inventor, (ii) the existence of working examples, and (iii) the quantity of experimentation needed to make or use the invention based on the content of the disclosure. See MPEP § 2164.01(a). Applicants submit that the art of record does not disclose each element of the claims now pending. In addition, Applicants submit that one prior art reference (Weiss) does not fully enable the Applicants' invention. Therefore, Applicants respectfully traverse this rejection.

a) Over Bruchez, et al. ("Bruchez")

Claims 1-26 are rejected as allegedly being anticipated by Bruchez (U.S. Pat. No. 6,274,323). The Examiner has cited Bruchez for disclosing a method of counting the presence of a single copy of a target nucleic acid in a sample. However, the cited reference fails to teach all of the claimed elements of the invention. In particular, Bruchez teaches ensemble detection.

Both Bruchez, in Example 1, and Applicants, on page 17, line 18, have conducted Qdot Immunosorbent Assays (QISA). In this assay, different amounts of biotinylated rabbit IgG were placed on a surface. The surfaces were washed with streptavidin functionalized quantum dots. Some of these streptavidin functionalized quantum dots bound to the biotinylated rabbit IgG. After the non-bound streptavidin functionalized quantum dots were washed away, the surfaces were imaged.

Applicants, in their QISA, applied 10 nM to 100 fM solutions of biotinylated rabbit IgG to the surface. As can be seen in Figure 3A, signal from quantum dot-bound single molecules was observed. As can be seen from the ordinate of the chart in Figure 3B, these single molecules were counted and their densities recorded across the observable range.

A similar experiment is disclosed in Bruchez, however, the results are in contrast with Applicants' results. In Bruchez's Figure 2, the ordinate displays the total emission (fluorescence) intensity of a population of the quantum dot-bound target species, which is a hallmark of ensemble detection, as explained in **The Invention** section above. Therefore, Bruchez does not expressly or inherently describe each and every element of claims 1-19 and 23-26. Applicants respectfully request withdrawal of the rejection.

b) Over Weiss ("Weiss")

Claims 1-3, 17, 19-21, and 23-26 are rejected as allegedly being anticipated by Weiss (U.S. Pat. No. 6,207,392). Weiss is cited for disclosing a method of counting the presence of a single copy of a target species in a sample.

(1) The cited reference fails to teach all of the claimed elements of the invention

Weiss fails to teach the element of resolving the optical characteristic of two quantum dots attached to a single molecule from quantum dots not attached to said single

molecule. This element was disclosed in Applicants' claims 20-22. The Examiner has cited Column 17, lines 11-31 of Weiss as disclosing this element. However, the first and second quantum dots referred to in the Examiner's citation are not attached to the same single molecule, but rather attached to a "first detectable substance" (Column 17, line 16) and a "second detectable substance" (Column 17, line 23), respectively. Furthermore, Applicants' "single molecule of a target nucleic acid" is not a part of Weiss's definition of a "detectable substance" (Column 6, lines 35-40). Since Weiss detected quantum dots attached to two detectable substances which are not single nucleic acid molecules, Weiss fails to teach every element of the Applicants' invention.

Applicants have incorporated the limitations of claims 20 and 21 into independent claims 1 and 17, respectively, and subsequently into claims 2-3, 19, and 23-26, which are dependent upon claim 1. Because Weiss does not teach the element of resolving the optical characteristic of the first and second quantum dot attached to a single molecule from an optical characteristic of a quantum dot not attached to the single molecule, Weiss does not disclose each and every element of claims 1-3, 17, 19, and 23-26. In the absence of a disclosure or suggestion of each claimed element, a *prima facie* case of anticipation cannot be set forth.

(2) The cited reference does not fully enable the Applicants' invention

The Weiss reference fails to enable one of skill in the art to make and use the claimed invention. In particular, since Weiss (i) does not provide direction for the important steps of single molecule counting, (ii) provides no working examples of single molecule counting, and (iii) requires a large amount of experimentation to make or use Applicants' invention, one of skill would be unable to make or use Applicants' method of single molecule counting, based upon the disclosure in Weiss, without undue experimentation.

(i) Weiss does not provide direction for the important steps of single molecule counting

As described in **The Invention** section above, single molecule counting can only be performed at a concentration or density where the quantum dot-bound single molecules can be spatially resolved. Furthermore, a detection system (such as a CCD camera or a laser scanning

confocal microscope) must be selected. Therefore, a reference describing single molecule counting must describe both an effective concentration or density range for the quantum dot-bound single molecules of interest and a detection system to view the results.

The Weiss reference does not describe either of these necessary steps for performing single molecule counting. Weiss does not disclose a concentration or density range for performing single molecule counting experiments. Additionally, while Weiss suggests that the signal from a quantum dot-linked detectable substance needs to be detected (column 4, lines 65-67; column 17, lines 45-48), there is no disclosure of the kind of detection system needed. Since there is no discussion of concentration/density ranges or detection systems, Weiss does not provide direction for the important steps of single molecule counting.

(ii) Weiss fails to provide working examples of single molecule counting

In the Examples section of the written description, Applicants disclose cases of single molecule counting. These Examples, starting on page 59, line 29, detail the experimental methods necessary to perform single molecule counting on glass coverslips. This includes such information as the amount of time required to label the single molecules with quantum dots, as well as the type of solution used to prepare the glass coverslips for imaging and to wash away unbound quantum dots.

There are two working examples, Example 1 and Example 2, in the Weiss reference. Both Example 1 and Example 2 provide experimental methods detailing the synthesis of quantum dot compounds. The Examples do not contain experimental methods for any use of the quantum dot compounds, let alone for single molecule counting. Therefore, Weiss fails to disclose working examples for single molecule counting.

(iii) Based upon the Weiss reference, a large amount of experimentation is required to make or use Applicants' invention

As mentioned above, the Weiss reference does not disclose concentration ranges, detection systems, or working examples necessary for single molecule counting. Therefore, one of skill in the art would have to perform a series of concentration-varying experiments with a variety of detection systems in order to discover Applicants' method of single molecule counting. Considering that the density range where single molecule counting cannot occur spans at least

five orders of magnitude (page 22, lines 15-19), a large amount of experimentation would be required, based upon Weiss, in order to make or use Applicants' invention.

Because Weiss does not provide direction for the important steps of single molecule counting, provides no working examples, and requires a large amount of experimentation to make or use Applicants' invention, undue experimentation is required to perform Applicants' invention based upon the disclosure of Weiss. Therefore, Weiss does not enable one of skill to make or use Applicants' invention. Thus, Weiss is not an anticipatory reference, and Applicants respectfully request withdrawal of the rejection.

Under 35 U.S.C. § 103(a)

In order to establish a *prima facie* case of obviousness, the rejection must demonstrate that (1) the cited references teach all the claimed elements; (2) there is a suggestion or motivation in the prior art to modify or combine the reference teachings; and (3) there is a reasonable expectation of success. MPEP § 2143; *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991). As explained below, the cited references fail to disclose all the elements of the claimed invention and fail to provide a basis for one of skill to either combine the references or reasonably expect that the references' methods would be useful for single molecule detection or resolution. Therefore, Applicants respectfully traverse the following rejections.

a) Over Bawendi et al. ("Bawendi") in view of Singer et al. ("Singer")

Claims 1-8, and 17-26 are rejected as allegedly being obvious over Bawendi (U.S. Patent No. 6,306,610) in view of Singer (U.S. Patent No. 5,866,331).

The Examiner has cited Bawendi for teaching a method of detecting the fluorescence of two distinguishable quantum dots attached to a target species immobilized on a substrate. The Examiner acknowledges that Bawendi differs from the claimed invention in that Bawendi does not teach single molecule counting. Singer is cited by the Examiner for allegedly teaching single copy counting.

(1) The cited references fail to teach all the claimed elements

The references cited by the Examiner fail to teach all of the claimed elements of the invention. In particular, none of the cited references teach the counting of quantum dot-bound single molecules.

Singer professes to detect a single probe bound to a target molecule. (Column 4, lines 45-49; Abstract, last sentence.) To accomplish this, however, Singer must attach at least five fluorochromes to each probe (Column 4, lines 49-51). In order to quantify the amount of the target species, Singer must: a) measure the Total Fluorescence Intensity (TFI) of all the fluorochromes (Column 6, lines 58-62); b) know the TFI per fluorochrome (Claim 1, part e)); and c) know the TFI per probe (Claim 1, part f)). Thus, Singer is detecting the intensity of a population of target species molecules, rather than counting an optical characteristic from quantum dot-bound individual molecules, as described in Applicants' invention.

Since neither Singer nor Bawendi teach the counting of quantum dot-bound individual molecules, the references fail to teach all of the claimed elements of the invention. In the absence of a disclosure or suggestion of each claimed element, a *prima facie* case of obviousness cannot be set forth.

(2) There is no suggestion or motivation to modify or combine the reference teachings

The references cited by the Examiner also fail to provide a suggestion or motivation to modify or combine the references. In particular, the two cited references teach the use of different numbers of fluorochromes. See Figure 1, attached hereto, for a comparison of the fluorochrome requirements of Bawendi and Singer. In Bawendi's method described above, two probes, each containing one quantum dot, are attached to a target species. Singer, on the other hand, requires at least five fluorochromes attached to each probe. In order to use Bawendi's two probe technique in concert with Singer's method, one would need to use no less than ten quantum dots. Since the two cited references provide conflicting information on their fluorochrome requirements, there is no motivation to combine the two. Thus, a *prima facie* case of obviousness cannot be set forth.

(3) The cited references do not provide a reasonable expectation of success

The references cited by the Examiner fail to provide a reasonable expectation of success in performing the Applicants' invention. As mentioned earlier, both Bawendi and Singer employ ensemble detection, which can involve conducting experiments at concentration or density ranges where individual molecules cannot be imaged. Since single molecule counting is very unlikely to occur at concentration or density ranges suitable for ensemble detection, Bawendi and Singer do not provide a reasonable expectation of success in performing the Applicants' method. Thus, a *prima facie* case of obviousness cannot be set forth.

The cited references teach away from the Applicants' invention

In addition to not meeting the criteria of the *prima facie* case, the cited references also teach away from the Applicants' invention. (See *In re Fine*, 837 F.2d 1071, 1074 (Fed. Cir. 1988) stating, "...[it is] error to find obviousness where references 'diverge from and teach away from the invention at hand.'" quoting *W.L. Gore and Assoc. v. Garlock, Inc.*, 721 F.2d 1540, 1550, 220 USPQ 303,311 (Fed. Cir. 1983)).

In Column 5, lines 49-57, Singer reports that,

> Despite the enhanced contrast and resolution of a restored image, **there is no way to determine the number of probes responsible for a particular point of fluorescence, unless the TFI of a single probe--in the imaging environment--is known.** The present invention provides a method of determining the TFI per fluorochrome (or per probe bearing a known number of fluorochromes) in an imaging environment. [emphasis added]

According to Singer, it is not possible to quantify the amount of a target species without knowing the TFI. However, Applicants' method is employed at low enough concentrations or densities that each point of fluorescence corresponds to a quantum dot-bound single molecule. This alleviates the need to know the TFI. Since Singer instructs that TFI must be known in order to quantify the amount of a target species, and Applicants have discovered a method that does not require this knowledge, Singer, in fact, teaches away from the Applicants' invention.

Because the cited references fail to teach all the claimed elements, do not contain a suggestion or motivation to modify or combine the reference teachings, and do not provide a

reasonable expectation of success, a *prima facie* case of obviousness cannot be set forth. In fact, the cited references specifically teach away from the methods in Applicants' invention. Thus, Applicants respectfully request withdrawal of the rejection.

b) Over Weiss and Söderlund et al. ("Söderlund") in view of Chan et al. ("Chan")

Claims 4-14, and 18 are rejected as allegedly being obvious over Weiss and Söderlund (U.S. Patent No. 6,013,431) in view of Chan (*Science* (1998) **281**: 2016-2018).

Weiss is described above. Söderlund is cited for teaching that numerous inherited diseases are caused by polymorphisms, and methods for detecting polymorphic regions are clinically important. Chan is cited for teaching that radioactive labels are hazardous and short-lived, while quantum dot labeling provides safe and durable labels which are extremely sensitive and DNA-attachable.

Applicants have incorporated the limitations of claims 20 and 22 into independent claims 1 and 18, respectively, and subsequently into claims 4-14, which are dependent from claim 1. Because the combination of Weiss, Söderlund, and Chan does not teach the element of resolving the optical characteristic of first and second quantum dots attached to a single molecule from an optical characteristic of a quantum dot not attached to the single molecule, the combination does not disclose each and every element of claims 4-14, and 18. Thus, a *prima facie* case of obviousness cannot be set forth.

Applicants respectfully request withdrawal of this obviousness rejection.

c) Over Weiss and Söderlund in view of Chan and further in view of Bawendi

Claims 15 and 16 are rejected as allegedly being obvious over Weiss and Söderlund in view of Chan and further in view of Bawendi. The references have been described above.

Applicants have incorporated the limitations of claim 20 into independent claim 1 and subsequently into claims 15 and 16, which are dependent from claim 1. Because the combination of Weiss, Söderlund, Chan, and Bawendi does not teach the element of resolving the optical characteristic of first and second quantum dots attached to a single molecule from an optical characteristic of a quantum dot not attached to the single molecule, the combination does

not disclose each and every element of claims 15 and 16. Thus, a *prima facie* case of obviousness cannot be set forth.

Under Obviousness-Type Double Patenting

a) Over Bruchez

Claims 1-3, and 17 are rejected under the judicially created doctrine of obviousness type double patenting over claims 1-26 of Bruchez. Since the issued patent was filed prior to the pending patent application, a one-way obviousness test is appropriate. MPEP § 804(II)(B)(1)(a). Thus, to maintain a double patenting rejection under the judicially created doctrine of obviousness-type double patenting, the Examiner must set forth a proper *prima facie* case of obviousness. The three criteria for a *prima facie* case of obviousness are described above, but are not met in this case. First, as mentioned earlier, Bruchez teaches ensemble detection, and therefore does not teach all of the claimed elements of the Applicants' invention. Second, as Bruchez's method teaches ensemble detection, which is not part of the Applicants' invention, there is no motivation to use Bruchez's method in the practice of Applicants' invention. Finally, since ensemble detection is performed in concentration or density ranges where single molecule detection is very unlikely, there is no reasonable expectation of success in employing Bruchez's method in the practice of Applicant's invention. As a proper *prima facie* case of obviousness cannot be made, Applicants submit that the instant rejection for double patenting is improper.

b) Provisional rejection of copending application 09/784,866

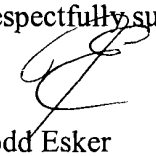
Claims 1-8 and 10 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-26 of copending U.S. Application Serial No. 09/784,866. As the rejection is provisional, none of the claims have yet been allowed. Therefore, Applicants request that the rejection be held in abeyance until one or more claims of the allegedly conflicting application are found allowable.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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